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ON THE GELATINIZATION BY HEAT OF WHEAT AND MAIZE STARCH

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When a suspension of starch granules in water is heated, the granules first swell, then burst, and finally lose their anisotropy, or double refraction. These successive changes in starch grains can be observed under the microscope as the original suspension is converted into a much more viscous product which is usually spoken of as a starch paste. The belief or assumption has prevailed quite generally that all changes which are involved in the conversion of a starch suspension into a paste take place at the so-called gelatinization temperature which has been regarded specific for each individual starch. Because of this supposed specificity, gelatinization temperatures have been recorded for the more common starches. These temperatures are frequently referred to in the consideration of starches, especially in the identification of unknown specimens. The literature is reviewed by E. T. Reichert (16).

Scrutiny of the reports on gelatinization temperatures indicates that neither the materials nor the methods used were sufficiently uniform to produce comparable results. Often the samples examined were those of commercial starches, which are almost invariably treated with acids or alkalis in some stage of their purification. Acids and alkalis have even been used in the preparation of laboratory or experimental samples. Such preparations have undoubtedly been denatured more or less and rendered unfit as experimental specimens, because starch has acid properties and a capacity for combining with alkalis. Rask and Alsberg (15) found that the hydrogen-ion concentration of wheat starch pastes is decidedly on the acid side of neutrality. Tadokoro and Sato (22) state that rice starches are electro-negative colloids.

Samec (18) states that alkalis have a pronounced influence on the effect of heat on starches. Wolff and Fernbach (25) report analogous observations.

LaWall and Graves (9), Nyman (13), and Reichert (16) state that the gelatinization temperature of a starch freshly isolated from living tissue is different from that of a starch which has been dried previous to gelatinization. Drying potato starch raises the gelatinization temperature from 59° to 65°. In the case of drier products, like grains, differences are probably not so great. According to Samec (18) the length of time a starch has been wet is also a factor. It is therefore not astonishing that investigators who examined commercial starches or who used strong reagents in the purification of their experimental preparations should not have obtained concordant results. Differences in methods of preparation were probably responsible for variations in gelatinization temperatures which Samec observed for different commercial potato starches.

The character of the raw material from which the starch is prepared may also determine some of its properties. Until recently investigators have assumed that the starch from a single botanical species is always the same. As a matter of fact, it has been shown definitely that the starches of different horticultural varieties of the same species are not necessarily identical. Whymper (24) found that wheat starches differ in their resistance to diastase. Stakman, Parker, and Piemeisel (21) and Leach (10) suggested that the varying resistance of different strains of wheat to rust may be due in part to differences in the structure of their starches. Collatz (1) found that the starch of strong flours appears to be more easily hydrolyzed by diastatic ferments than that of weaker flours. Rask and Alsberg (15) found that the viscosities of the gelatinized starches of different varieties of wheat differ greatly. LaWall and Graves (9) reported the temperatures of gelatinization of different varieties of bean and pea starches to be different, while Fernbach and Wolff (5) have found that the starch of immature garden peas (variety not given) is unique in that it behaves like "reverted starch," such as is obtained by letting starch solutions stand at low temperatures. Whymper (24) found that the temperature of gelatinization varies with the state of maturity of the starch granules. It has been known for some time that starch grains of wrinkled garden peas are compound while those of smooth varieties are simple (8, 3). Dox and Roark (4) found different gelatinization temperatures for the starches of different varieties of maize, and Francis and Smith (7) made the same observation on

sorghums. It is well known that the starch of certain varieties of cereals gives a red color with iodine, and that of other varieties a blue color. This is true of millet and rice, perhaps also of corn (23). Tadokoro and Sato (22) have found that rice starch that is colored red with iodine gives more viscous pastes than that colored blue, and this seems to be true as well in the case of millet. There is, then, abundant evidence that the starches of different varieties of the same species are not identical. This fact probably accounts for some of the discrepancies in the recorded temperatures of gelatinization as suggested by Dox and Roark (4) and by Reichert (16).

A further reason for the discrepancies in the results of different investigators is to be sought in the methods used for the determination of the gelatinization points. Three types of method have been used: (1) Observation of the granules under the miscroscope; (2) observation of changes in the translucency of the starch suspensions as the temperature is raised; (3) changes in viscosity as the temperature is raised.

The objection to the method of fixing the gelatinization temperature by observing the granules under the microscope is that it permits too much play for personal judgment. This was recognized by E. Lippmann (11), who made the first exact study of the gelatinization temperature of different starches, for he recorded the temperature at which individual grains begin to swell, the temperature at which individual grains burst and disintegrate, and the temperature at which gelatinization is complete. The interval between the first and the last temperature ranged from 4 degrees in the case of arrowroot to 30 degrees in the case of acorn starch. Moreover, the rate at which the temperature of the suspension is raised seems to be not without influence (13, 4, 7, 12). Nyman (13) took as the temperature of gelatinization the point at which the double refraction of the larger grains disappears. Reichert (16) and also LaWall and Graves (9) took as the gelatinization temperature the point at which anisotropy disappears from the majority of the grains; while Francis and Smith (7) took the point at which it disappears in all the grains. Dox and Roark (4) took the temperature at which anisotropy disappears in all grains large enough to show the characteristic shape and markings. It is therefore evident that the microscope method leaves a wide spread for the exercise of judgment. Moreover, in the same starch preparation the behavior of the grains of different size is different (12). According to Whymper (24) and Nyman (13) the larger grains of any given starch gelatinize at a lower temperature than the smaller ones.

Nyman suggested that this might be due to the fact reported by Lenz[1] that the membranes of the larger grains are thinner than those of the smaller ones. Some starches, however, exhibit the opposite behavior (16), the smaller grains gelatinizing at a lower temperature than the larger ones.

The method based on the change of translucency was originated by Samec (18). An incandescent lamp bulb is immersed in the starch suspension contained in a glass vessel. As the vessel is heated, a very sharp change in translucency is observed. This effect is not to be secured by long-continued heating at a temperature lower by 1° to 2°.

The viscosity of starch pastes has been studied by several investigators. Ostwald (14) has suggested the possibility of employing a viscosimetric technique to determine the so-called gelatinizing point of starches. His technique consists in determining the viscosities of a suspension of the starch over a temperature range which will include the gelatinizing region. According to Ostwald gelatinization will be shown by a sudden and sharp increase in the viscosity of the suspension. The temperature at which this increase takes place is to be regarded as the gelatinizing point.

To the writers it seemed that the viscosimetric method, but little used hitherto, might prove more valuable than the others for the determination of gelatinization temperatures. It also seemed to them important to examine starches that had never been subjected to the action of alkali or other chemically active reagent. It was thought that with such starch, using the viscosimetric method, sharp reproducible determinations of gelatinization temperatures might be made and that these might prove characteristic of different types of starches.

Accordingly a series of wheat starches was prepared with the use of only one per cent sodium chloride solution, water, alcohol, and ether. The method has been described in detail in an earlier paper (15). For purposes of comparison a sample of commercial corn (maize) starch that had been washed and dried was also examined. The viscosities of suspensions of these starches varying in concentration from 3 to 10 per cent were traced through temperatures ranging from 25° to above 90°. The viscosimeter employed was that of Stormer. The starch suspended in water of room temperature was placed in the cup of the instrument. The bath was heated by means of a microburner at such a rate that the temperature of the starch suspensions was raised gradually and uniformly from 25° to above 90° in the course of 40 to 45 minutes. During this rise in temperature, viscosity

[1] Lenz, —. Arbeiten aus dem Pharmazeutischen Institute, Berlin. Cited by Nyman (13).

determinations were made at every one or two degrees rise. In this manner the viscosities of eighteen or twenty suspensions of different starches were determined in varying concentrations. The results of all of these need not be reported. Suspensions of commercial corn and marquis wheat starches of 4½% and 5% concentrations have been selected as typical. The data upon these are given in the following table and presented graphically in the form of curves. In recording these results, stop-watch readings (in fifth seconds) were used as viscosity units, because they show better than any conversion units the changes as actually observed in the experiments, and thereby are indicated the degree of accuracy and sensitiveness of the technique.

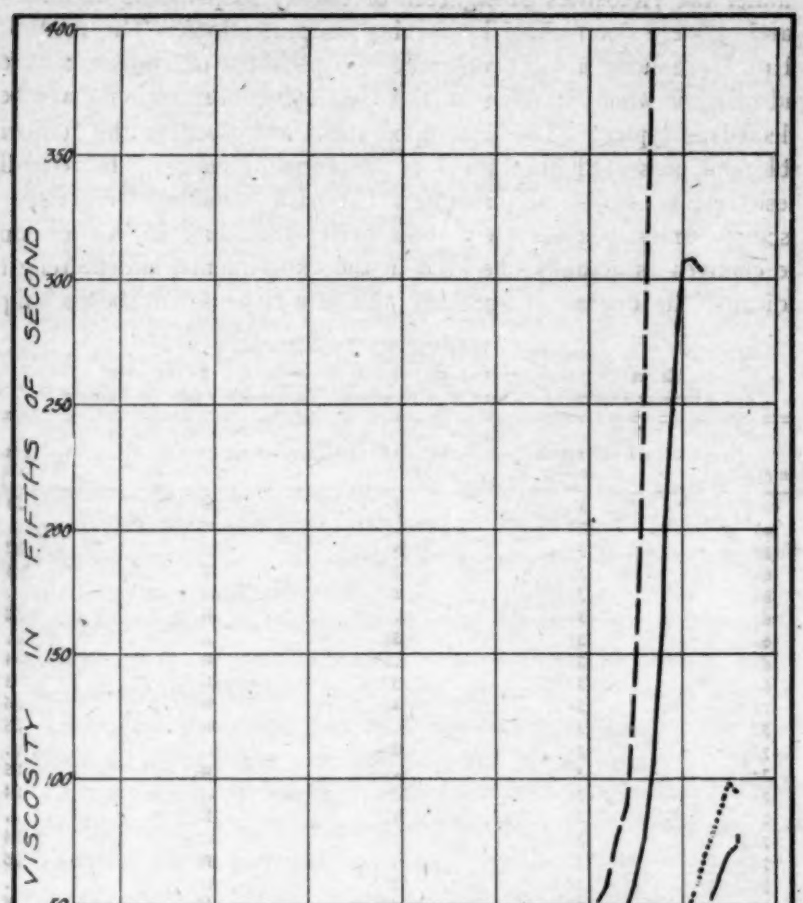
GELATINIZATION OF STARCH

EFFECT OF TEMPERATURE ON VISCOSITY OF STARCH SUSPENSIONS

Viscosity expressed as time in fifth seconds required for 100 revolutions

Temperature	4-½% Concentration		5% Concentration	
	Corn starch Viscosity	Wheat starch Viscosity	Corn starch Viscosity	Wheat starch Viscosity
25	33	33	34	34
40	32	..	33	..
50	31	33	31	32
60	31	32	31	33
62	31	31	..	..
63	31	..	31	32
65	31	34	..	..
66	31	..	31	34
68	33	33	34	35
70	34	33	35	36
72	34	..	36	35
73	34	34	..	..
74	34	..	38	35
75	35	35	..	34
76	36	..	40	..
77	37	36	..	34
78	37	..	40	36
79	37	35	42	..
80	37	35	46	36
81	38	..	51	37
82	40	35	58	37
83	44	36	75	37
84	46	..	92	37
85	59	..	143	38
86	78	36	265	39
87	108	..	432	40
88	164–202	38	647	43
89	239	..	894	44
90	296–307	41	1056	46
91	308	..	1009	51
92	304	43	....	64
93	...	50	....	72
94	...	62	....	87
95	...	71	....	98
96	...	75–79–78	....	96

Gelatinization of Starches[1]



[1] Data from Table

It will be noted that up to 65° there is no significant change in the viscosities of the suspensions. There is a slight decrease but this is probably due entirely to the water, the viscosity of which decreases similarly under these conditions. At 65° to 68° the viscosities begin to increase very gradually. However, as shown by the curves, there

is also uniform increase in the rate of increase of viscosity with respect to temperature until maximum viscosities are reached at 91° in the corn starch and 95° in the wheat starch. These increases in viscosity are obviously due to simultaneous and therefore correspondingly gradual changes in the starch grains, changes which extend over a range of 25° to 30°.

The exact changes which take place are not known, but they undoubtedly involve the starch grains in imbibition of water and consequent swelling to a point at which the grains burst. The final result is a conversion of the starch suspension into a sol which gels on cooling. This conversion may be defined as gelatinization. The temperature at which anisotropy disappears in starch grains can only mark an early stage of the gelatinization process which, as shown by the curves, extends over a range of 25° to 30° and makes its greatest progress at temperatures 20° or more above that at which gelatinization begins. The so-called gelatinization temperature, therefore, must not be regarded as the temperature at which gelatinization is completed but only that of an early stage of the process, for Mayer (12) found changes in the appearance of starch grains under the microscope before the disappearance of anisotropy, while LaWall and Graves (9) observed changes on warming starch suspensions at 37° for thirty minutes. Samec (18) made the comment that starch suspensions became more translucent before corresponding changes could be observed under the microscope. His statement that his translucency method determines only one stage of the gelatinization process would seem to imply that he regarded this process as a continuous one, altho he does not say so specifically. A similar view is implied in a general way by Mayer. Such conceptions of the gelatinization process are in harmony with the conclusion that must be drawn from the observations reported in this paper. It is that gelatinization is not a sharp transition point like the melting points of crystalline substances. It resembles more nearly the gradual softening processes of glass and other non-crystalline substances which show no definite melting points. This resemblance suggests the probability that heat in the presence of water softens starch in a like manner. As this gradual softening process begins at a rather indefinite and variable temperature and extends over a range of 20° to 25°, it seems inadvisable to record any definite gelatinization temperatures and certainly not to .01 of one degree, as Reichert and others have done. Obviously such records are deceptive. The conclusion to be drawn from these observations and considerations is that gelatinization is not a sharp transition point but

a long and gradual conversion process which may or may not begin at the so-called gelatinization temperature.

The conception that gelatinization is a gradual process is consistent with the disappearance of anisotropy in the early stages of gelatinization. Czapek (2) has suggested that the dark cross of the starch grain may be due to symmetrically distributed tensions [cf. also Sponsler (20)], for Fischer (6) observed the dark cross phenomenon in the hardened mucilage of certain orchids. Moreover, a drop of gelatin gel when dried shows double refraction, the shortest optical axis being parallel to the direction in which the drop has contracted (6, 19).[2] This double refraction may be due to the formation of internal strains as a result of the drying process. If this assumption is sound, it is more than probable that the anisotropy of the starch grain is the result of strain, and that in gelatinization anisotropy disappears because warming removes strains. Its disappearance in that event need not necessarily mean that any material change has as yet taken place in the starch molecules. The process might be regarded as quite analogous to annealing, except that water must be present. Heating to 100°C in glycerine according to Francis and Smith (7) does not cause anisotropy to disappear. In this respect starch resembles certain protein gels first studied by Rollett (17). If prepared from concentrated solutions, the gels do not melt on warming. However, if they be permitted to swell in water before warming, they melt. Possibly the gelatinization of starch is to be regarded in this wise: The starch gel first imbibes water. In this process strains are presumably removed and anisotropy disappears. When the starch gel has thus reached a certain degree of dilution, it melts. If this is the course of events, it is not necessary to assume, as has been done so frequently, that the gelatinization of starch depends upon depolymerization. The conception of gelatinization here presented is concordant with certain observations that are not readily explainable on the assumption that starch during gelatinization depolymerizes, using this term in the strictly chemical sense. These observations are: that sharply drying starch or thoroly wetting it (13) changes the gelatinization temperature; that gelatinization is incomplete unless adequate quantities of water are present (7); and that the rate of heating has a marked effect upon the gelatinization temperature (7).

Summary

In the process of gelatinizing wheat starch by heating suspensions in water, the viscosity increases gradually over a range of 25 to 30 degrees. There is therefore in all probability no definite temperature

[2] Quincke, quoted by Sheppard and Elliott (19).

of gelatinization. If the temperature of gelatinization be defined as that at which anisotropy disappears, then it must be regarded as marking the temperature of an early stage of the gelatinization process, not the temperature at which the process is completed.

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WHEAT AND FLOUR STUDIES. II. AGING I. THE CHANGE IN HYDROGEN-ION CONCENTRATION OF WHEAT AND MILL PRODUCTS WITH AGE

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Introduction

Jessen-Hansen (14) emphasized the importance of the hydrogen-ion concentration of the dough in yeast fermentation when he found that the most desirable loaf was obtained at a pH of approximately 5 and that if the dough was less acid than this, improvement could be obtained by adding sufficient acid to produce such a concentration.

Henderson, Fenn and Cohn (13) found that the viscosity of a dough passed through a minimum at a pH of a little below 5. They believe that one of the advantages of bringing a dough to a pH of 5 before baking is to be attributed to the minimum viscosity of the dough at this hydrogen-ion concentration.

Rumsey (16) found the optimum hydrogen-ion concentration for flour diastase to be at a pH of approximately 4.7, while Collatz (11) found the optimum for malt diastase to be in the neighborhood of this same hydrogen-ion concentration.

Euler and Emberg (12) found that yeast showed its maximum activity at approximately a pH of 5.

The author found the hydrogen-ion concentration of minimum solubility of gluten to lie in the region from pH 5.0 to 5.3.

Balland and Mosson (8) pointed out that the rope organism, if present in bread, could not withstand the combined effect of acidity and heat. Cohn, Walbach, Henderson, and Cathcart (10) and Morrison and Collatz (15) have associated the acidity necessary to prevent growth of rope in bread with a pH of 5.

The investigations cited indicate the desirability of carrying out yeast fermentation in the region of pH 5. The investigations of Collatz (11) Bailey and Sherwood (7) and unpublished observations by the author have shown very definitely that the hydrogen-ion concentration of the dough grows more acid as the fermentation progresses and that with some flours a pH of 5 or even a little less is reached by the dough at the time it goes into the oven. As some doughs never reach this hydrogen-ion concentration during normal fermentation, it would seem that they are not baked under the optimum condition. Jessen-Hansen

[1] Published with the approval of the Director.

(14) states that the hydrogen-ion concentration of flour increases with age and that the beneficial effect of aging is to increase the hydrogen-ion concentration toward the optimum of a pH of 5. Bailey and Johnson (4) have demonstrated the increase in the hydrogen-ion concentration of flours with age.

Alsberg (1) states: "It has been pretty definitely shown that the main cause of the maturing of flour is the development in it of a proper degree of acidity."

Bailey and Le Vesconte (5) investigated the extensibility of doughs, using the Chopin (9) extensimeter. They conclude that this instrument gives an indication of strength or baking value of flour. The higher extensimeter readings correspond to the flours of greater strength. Upon investigating the relation of hydrogen-ion concentration to extensibility of dough, they found the maximum to occur at a pH of approximately 6.0. A falling off in either direction occurred, so that at pH 5.0 the reading was less than 80 per cent of the reading at pH 6.0. They also found the extensimeter reading decreased as fermentation progressed.

In view of the importance which seems to be attached to producing a certain acidity in dough and of the fact that flours are known to grow more acid with age, not alone in titrable acidity but also in actual hydrogen-ion concentration, and that the beneficial effect of aging on flour has been attributed to this increase in acidity, it seems well to inquire into the factors which influence the rate of this change. The factors which one would naturally think of as being of the most importance are moisture content, temperature, and grade. In this connection Tague (19) reports an increase in hydrogen-ion concentration of the flour due to tempering, amounting in some experiments to 0.2 of a pH unit.

Experimental

Two wheats of the Marquis variety were chosen for this investigation. The first was a normal sample which had been grown on the plots of the experiment station. The second was sown late on an adjacent plot and as a result was frosted in the dough stage. This sample was immature and showed 30 per cent frost damage. These wheats were both milled in a small experimental mill and divided into the following mill products: a very long patent flour, a low-grade flour, shorts, and bran. In order to give some idea of these separations the ash content of each was determined. The yield of product is expressed on the moisture-free basis as is also the ash content. The results are given in Table I.

TABLE I

ASH IN WHEAT AND MILL PRODUCTS CALCULATED ON THE MOISTURE-FREE BASIS

	Normal wheat			Frosted immature wheat		
	Yield of mill products dry basis	Ash	Ash from 100 grams	Yield of mill products dry basis	Ash	Ash from 100 grams
	%	%	gms.	%	%	gms.
Wheat	100.0	1.85	.....	100.0	2.12	.....
Long patent flour	60.1	0.55	.331	48.9	0.83	.406
Low-grade flour..	11.6	0.91	.106	10.2	1.39	.142
Shorts	15.8	4.19	.662	24.8	3.71	.920
Bran	10.9	6.59	.718	12.7	4.80	.610
Total	98.4	....	1.817	96.6	....	2.078

The yield of flour in the frosted sample is much lower while the shorts portion is higher than in normal wheat. The ash is more uniformly distributed throughout the kernel in the frosted sample. This tendency to a uniform distribution of the ash is attributed to the immaturity of the sample and not to the freezing. The protein content of each mill product as well as the grams of protein per 100 grams of wheat appearing in each fraction is given in Table II.

TABLE II

CRUDE PROTEIN (N×5.7) IN WHEAT AND MILL PRODUCTS CALCULATED ON THE MOISTURE-FREE BASIS

	Normal wheat			Frosted immature wheat		
	Yield of mill products, dry basis	Crude protein	Protein in 100 gms. of wheat	Yield of mill products, dry basis	Crude protein	Protein in 100 gms. of wheat
	%	%	gms.	%	%	gms.
Wheat	100.0	16.02	.....	100.0	14.70	.....
Long patent flour	60.1	15.30	9.195	48.9	13.50	6.602
Low-grade flour..	11.6	15.40	1.786	10.2	14.45	1.474
Shorts	15.8	18.75	2.963	24.8	16.95	4.204
Bran	10.9	18.40	2.006	12.7	16.06	2.040
Total	98.4	.....	15.950	96.6	.....	14.320

In addition to the study of the aging of these mill products, the aging of the whole wheat and of the ground wheat was also investigated. The plan of the experiment was to maintain samples at three temperatures: (1) Outside temperature; (2) room temperature, which averaged about 22°C.; and (3) 35°C., this last temperature being obtained by keeping the samples in an air thermostat electrically maintained at that temperature. Each sample was aged at three different moisture contents: (1) 4 to 6 per cent, (2) the natural moisture content of the sample, and (3) about 15 per cent. The original moisture content of the wheat was about 9 per cent. The original moisture content of the mill products was influenced by tempering and the loss of moisture during milling. The lowest moisture content was obtained

by placing enough of each product to give three aliquots in a vacuum oven over sulphuric acid, the temperature being held at 30°C. When the samples had reached about the proper moisture content as shown by the loss in weight determined on a rough balance, each sample was divided into three parts for storing at the three different temperatures. At this time a more accurate moisture determination was made on duplicate 5-gram portions. The highest moisture contents were obtained for the whole wheat by adding the calculated amount of distilled water; and for the mill products by maintaining them in an atmosphere saturated with water vapor until the increase in weight of the material indicated that the desired moisture content had approximately been reached. The samples were then divided each into three parts for storing at the three temperatures, and more accurate moisture determinations made. It is unfortunate that the various products did not all reach the same moisture content. They were not all treated at once in the same container so conclusions as to the relative affinity for water can not be drawn from the data given. Bringing two different products to exactly the same moisture content in such a manner that the method of reaching it will not affect their subsequent behavior, presents considerable difficulty, especially when the adjustment must take place in limited time. The samples were stored in glass bottles of about 130 cc. capacity. They were closed with cork stoppers, paraffined. The total number of samples studied was 108.

The extracts from which the concentration of hydrogen ions was determined, were prepared by adding 25 cc. of distilled water to 5 grams of the sample and allowing it to stand, with occasional shaking, for 1 hour. The determinations of the hydrogen-ion concentrations of the mixtures were carried out using a Bailey (2) electrode and a Leeds and Northrup type K potentiometer and type R galvanometer. The normal calomel half cell was used and the millivolt readings were converted to pH values by means of the Schmidt and Hoagland (17) tables. In weighing out the samples the differences in moisture content were taken into consideration so that in all cases the weight taken represented 5 grams at the original moisture content of each product. The hydrogen-ion concentrations were determined at four-week intervals during the early part of the aging experiment and at longer intervals toward the end. The experiments started December 20, 1922, and extended over one year. The outside temperature was, of course, not constant. The samples were placed outdoors in the shade. On summer days these samples would undoubtedly reach a temperature considerably higher than the set stored in the laboratory. The determi-

nations were all made the same day and, in general, were not run in duplicate. Occasionally when duplicates were made they were found to check within one millivolt. The apparatus was tested out at the start and at the end of the run with a standard solution of known hydrogen-ion concentration. The results obtained are presented in Tables III to VIII.

The growth of micro-organisms which occurred in some samples, was usually first detected by odor and the appearance of these organisms was usually accompanied by a change in the hydrogen-ion concentration. This change was not always in the same direction and sometimes, as the moldy sample aged, the direction of the change would reverse.

Table IX presents an interesting relationship between the hydrogen-ion concentration of the original wheat and that of the mill products.

The normal wheat flours show a higher hydrogen-ion concentration than the original wheat while the shorts and bran show a lower. Jessen-Hansen (14) stated that the low-grade flours also had a lower hydrogen-ion concentration than the high grade flours. Bailey and Peterson (6) show that flours of different grade also have different hydrogen-ion concentrations. In the case of the frosted immature wheat the relationship is apparently not the same, but from other data it is probable that the determinations of the hydrogen-ion concentration of the wheat and long patent flour are too high. Other results on hand, however, indicate that the hydrogen-ion concentration of the mill products from immature wheat are more nearly the same than with mature wheat.

The results obtained for the changes in hydrogen-ion concentration of the wheat kernel are at first very erratic. This may be due to differences in the fineness to which the sample was ground and the length of extraction. Beginning with the sixteenth week the samples were run through the grinding mill twice and were extracted for two hours instead of one hour. The effect of time and temperature of extraction on the hydrogen-ion concentration of the extract from ground wheat has been investigated by Swanson and Tague (18). They found that the shorter periods of extraction and lower temperatures tended to give solutions of higher pH, but that as the temperature and time were increased a lower limit was reached.

TABLE III

Wheat Kernels

Changes in hydrogen-ion concentration with age of wheat kernels from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the wheat containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
		Normal wheat								
Moisture, %		4.8	9.3	14.1	4.8	9.3	14.1	4.8	9.3	14.1
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.40	6.40	6.40	6.40	6.40	6.40	6.40	6.40	6.40
January 17	4	6.52	6.50	6.54	6.53	6.52	6.51	6.54	6.52	6.52
February 14	8	6.36	6.38	6.42	6.39	6.42	6.37	6.41	6.37	6.30
March 14	12	6.48	6.44	6.50	6.47	6.48	6.47	6.49	6.46	6.37
April 11	16	6.40	6.40	6.46	6.43	6.43	6.43	6.46	6.40	6.28
May 9	20	6.42	6.38	6.47	6.40	6.44	6.38	6.45	6.37	6.28
July 5	28	6.43	6.41	6.43	6.43	6.43	6.41	6.43	6.36	6.21
December 20	52	6.43	6.41	6.36	6.45	6.40	6.32	6.43	6.40	6.26
		Frosted immature wheat								
Moisture, %		5.9	8.8	14.8	5.9	8.8	14.8	5.9	8.8	14.8
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55	6.55
January 17	4	6.59	6.59	6.58	6.58	6.57	6.57	6.57	6.57	6.62
February 14	8	6.50	6.44	6.45	6.46	6.47	6.46	6.47	6.43	6.42
March 14	12	6.51	6.54	6.52	6.53	6.51	6.48	6.51	6.51	6.37
April 11	16	6.49	6.47	6.47	6.46	6.46	6.44	6.45	6.32	6.27
May 9	20	6.46	6.50	6.46	6.49	6.44	6.43	6.46	6.46	6.24
July 5	28	6.49	6.50	6.43	6.51	6.48	6.33	6.46	6.44	6.21
December 20	52	6.49	6.46	6.40	6.48	6.43	6.07	6.42	6.38	6.06

*Indicates evidence of the growth of micro-organisms.

TABLE IV

GROUND WHEAT

Changes in hydrogen-ion concentration with age of ground wheat from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the ground wheat containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
Normal wheat										
Moisture, %		4.3	9.1	16.0	4.3	9.1	16.0	4.3	9.1	16.0
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.40	6.40	6.41	6.40	6.40	6.41	6.40	6.40	6.41
January 17	4	6.41	6.40	6.33	6.42	6.36	6.19	6.40	6.35	6.10
February 14	8	6.39	6.33	6.21	6.36	6.31	5.85	6.31	6.24	5.82
March 14	12	6.34	6.37	6.22	6.38	6.31	5.73*	6.31	6.28	5.80*
April 11	16	6.34	6.36	6.22	6.39	6.31	5.63*	6.34	6.29	5.61*
May 9	20	6.32	6.34	6.17	6.37	6.32	5.53*	6.35	6.23	5.51*
July 5	28	6.39	6.33	5.51*	6.40	6.33	5.25*	6.35	6.23	6.40*
December 20	52	6.34	6.18	7.58*	6.39	6.23	5.27*	6.19	6.00	....
Frosted immature wheat										
Moisture, %		4.5	9.0	15.4	4.5	9.0	15.4	4.5	9.0	15.4
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.55	6.55	6.44	6.55	6.55	6.44	6.55	6.55	6.44
January 17	4	6.49	6.52	6.32	6.50	6.47	6.18	6.48	6.43	6.77*
February 14	8	6.46	6.43	6.20	6.42	6.39	6.03	6.39	6.34	7.20*
March 14	12	6.46	6.48	6.19	6.46	6.42	5.99	6.41	6.37	7.32*
April 11	16	6.45	6.43	6.15	6.43	6.40	5.93	6.39	6.31	7.08*
May 9	20	6.48	6.40	5.97	6.42	6.40	5.79*	6.39	6.30	7.00*
July 5	28	6.40	6.37	5.35	6.41	6.35	5.48*	6.34	6.28	7.10*
December 20	52	6.40	6.26	7.17*	6.36	6.29	5.37*	6.30	6.24	....

*Indicates evidence of the growth of micro-organisms.

TABLE V

LONG PATENT FLOUR

Changes in hydrogen-ion concentration with age of long patent flour from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the flour containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
		Normal wheat								
Moisture, %		5.3	12.2	14.4	5.3	12.2	14.4	5.3	12.2	14.4
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.14	6.14	6.16	6.14	6.14	6.16	6.14	6.14	6.16
January 17	4	6.12	6.13	6.07	6.10	6.11	6.01	6.08	6.01	5.81
February 14	8	6.12	6.08	5.99	6.04	6.01	5.94	5.98	5.90	5.61
March 14	12	6.07	5.98	6.00	5.99	6.05	5.90	6.05	5.91	5.48
April 11	16	6.25	6.26	6.14	6.05	6.05	5.82	6.03	5.91	5.23*
May 9	20	6.06	5.89	5.98	6.05	6.01	5.63	6.03	5.81	4.79*
July 5	28	6.13	6.06	5.94	6.08	6.01	4.82*	6.09	5.92	4.86*
December 20	52	6.06	5.99	4.72*	6.04	5.98	4.42*	6.01	5.81	....
		Frosted immature wheat								
Moisture, %		5.7	11.3	16.3	5.7	11.3	16.3	5.7	11.3	16.3
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.49	6.49	6.41	6.49	6.49	6.41	6.49	6.49	6.41
January 17	4	6.43	6.43	6.30	6.39	6.37	6.15	6.38	6.26	5.97
February 14	8	6.33	6.30	6.16	6.31	6.27	4.91*	6.31	6.12	5.75
March 14	12	6.38	6.34	6.20	6.37	6.29	4.85*	6.28	6.06	5.75*
April 11	16	6.35	6.33	6.15	6.37	6.22	4.62*	6.24	5.96	5.77*
May 9	20	6.33	6.28	5.91	6.35	6.19	4.86*	6.16	5.86	5.64*
July 5	28	6.34	6.25	6.01*	6.28	6.16	5.17*	5.94	5.66	5.55*
December 20	52	6.19	6.06	6.50*	6.21	5.99	5.27*	5.81	5.59	....

*Indicates evidence of the growth of micro-organisms.

TABLE VI

Low-Grade Flour

Changes in hydrogen-ion concentration with age of low-grade flour from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the flour containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
Normal wheat										
Moisture, %		4.9	10.9	16.4	4.9	10.9	16.4	4.9	10.9	16.4
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.33	6.33	6.33	6.33	6.33	6.33	6.33	6.33	6.33
January 17	4	6.29	6.26	6.22	6.29	6.24	5.82	6.26	6.19	6.60*
February 14	8	6.23	6.23	6.11	6.22	6.20	6.35*	6.19	6.08	6.49*
March 14	12	6.26	6.25	6.13	6.28	6.21	4.93*	6.20	6.04	4.00*
April 11	16	6.10	6.05	6.05	6.29	6.22	4.66*	6.24	6.11	6.56*
May 9	20	6.23	6.22	6.10	6.24	6.19	4.66*	6.24	5.98	6.35*
July 5	28	6.31	6.17	5.67*	6.08	6.20	4.58*	6.25	6.02	6.41*
December 20	52	6.22	6.13	5.27*	6.24	6.09	4.61*	6.10	5.82	....
Frosted immature wheat										
Moisture, %		4.1	9.6	15.0	4.1	9.6	15.0	4.1	9.6	15.0
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.49	6.49	6.44	6.49	6.49	6.44	6.49	6.49	6.44
January 17	4	6.45	6.47	6.32	6.46	6.44	6.10	6.32	6.37	5.97
February 14	8	6.42	6.40	6.18	6.37	6.32	5.12*	6.33	6.27	5.62
March 14	12	6.39	6.42	6.20	6.42	6.36	4.77*	6.32	6.24	5.71*
April 11	16	6.36	6.35	6.13	6.34	6.31	4.58*	6.29	6.03	5.41*
May 9	20	6.41	6.32	6.15	6.34	6.31	4.68*	6.33	5.97	5.20*
July 5	28	6.37	6.34	5.72*	6.36	6.27	4.79*	6.25	5.92	5.75*
December 20	52	6.26	6.20	4.99*	6.28	6.15	4.84*	6.12	5.90	....

*Indicates evidence of the growth of micro-organisms.

TABLE VII

SHORTS

Changes in hydrogen-ion concentration with age of shorts from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the shorts containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
		Normal wheat								
Moisture, %		4.8	11.9	14.1	4.8	11.9	14.1	4.8	11.9	14.1
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.48	6.48	6.45	6.48	6.48	6.45	6.48	6.48	6.45
January 17	4	6.48	6.47	6.39	6.49	6.47	6.26	6.50	6.32	5.99
February 14	8	6.46	6.43	6.27	6.46	6.38	6.05	6.40	6.23	5.68
March 14	12	6.44	6.41	6.26	6.45	6.37	5.94	6.44	6.22	5.63
April 11	16	6.49	6.43	6.23	6.45	6.38	5.67*	6.44	6.21	5.58
May 9	20	6.44	6.32	6.22	6.39	6.36	5.45*	6.46	6.23	5.24*
July 5	28	6.44	6.37	6.45*	6.34	6.28	5.14*	6.39	6.12	5.51*
December 20	52	6.40	6.17	7.10*	6.40	6.11	5.09*	6.34	6.02	....
		Frosted immature wheat								
Moisture, %		6.6	11.6	14.8	6.6	11.6	14.8	6.6	11.6	14.8
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.47	6.47	6.37	6.47	6.47	6.37	6.47	6.47	6.37
January 17	4	6.43	6.44	6.27	6.40	6.38	6.08	6.40	6.13	5.88*
February 14	8	6.35	6.34	6.07	6.34	6.25	5.85	6.29	5.99	6.99*
March 14	12	6.37	6.38	6.12	6.36	6.29	5.86*	6.33	6.00	6.49*
April 11	16	6.30	6.31	6.01	6.32	6.19	5.91*	6.26	5.86	6.17*
May 9	20	6.30	6.35	6.01	6.34	6.20	5.75*	6.25	5.89	5.95*
July 5	28	6.28	6.22	5.63	6.29	6.16	5.65*	6.19	5.87	5.83*
December 20	52	6.20	6.10	5.59	6.22	6.07	5.48*	6.14	5.79	....

*Indicates evidence of the growth of micro-organisms.

TABLE VIII

Bran

Changes in hydrogen-ion concentration with age of bran from normal and from frosted immature wheat; the aging, extending from December 20, 1922, to December 20, 1923, taking place at different temperatures and the bran containing different amounts of moisture

Temperature		Outside temperature			Room temp., about 22°C.			Thermostat 35°C.		
		Normal wheat								
Moisture, %		5.4	13.6	14.6	5.4	13.6	14.6	5.4	13.6	14.6
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.62	6.62	6.60	6.62	6.62	6.60	6.62	6.62	6.60
January 17	4	6.62	6.60	6.56	6.60	6.53	6.48	6.64	6.46	6.28
February 14	8	6.63	6.57	6.54	6.59	6.58	6.46	6.63	6.14	6.03
March 14	12	6.58	6.55	6.51	6.55	6.50	6.45	6.56	6.18	5.91
April 11	16	6.60	6.55	6.50	6.62	6.50	6.43	6.57	6.17	5.62
May 9	20	6.56	6.56	6.52	6.59	6.48	6.49	6.61	6.25*	5.72*
July 5	28	6.60	6.49	6.32	6.61	6.50	....	6.56	6.07*	5.87*
December 20	52	6.54	6.37	6.07	6.56	6.43	....	6.49	6.03*	....
		Frosted immature wheat								
Moisture, %		5.1	13.0	15.7	5.1	13.0	15.7	5.1	13.0	15.7
Date	Time, weeks	pH	pH	pH	pH	pH	pH	pH	pH	pH
December 20	0	6.47	6.47	6.46	6.47	6.47	6.46	6.47	6.47	6.46
January 17	4	6.42	6.46	6.35	6.46	6.39	6.21	6.41	6.04	5.92
February 14	8	6.40	6.34	6.21	6.35	6.28	6.00	6.34	5.92	5.99*
March 14	12	6.43	6.42	6.28	6.44	6.34	5.64*	6.40	6.03	6.97*
April 11	16	6.38	6.30	6.18	6.36	6.25	5.31*	6.33	5.91	6.63*
May 9	20	6.44	6.34	6.22	6.38	6.29	6.08*	6.39	5.93	6.18*
July 5	28	6.41	6.33	6.01	6.40	6.24	8.04*	6.31	5.96	5.85*
December 20	52	6.29	6.15	5.75	6.35	6.12	....	6.23	5.89	....

*Indicates evidence of the growth of micro-organisms.

TABLE IX

HYDROGEN-ION CONCENTRATION OF VARIOUS MILL PRODUCTS

	Normal wheat	Frosted immature wheat
	pH	pH
Whole wheat	6.40	6.55
Long patent flour	6.14	6.49
Low-grade flour	6.33	6.49
Shorts	6.48	6.47
Bran	6.62	6.47

There is no doubt that both increased temperature and increased moisture content accelerate the rate of increase in hydrogen-ion concentration. There seems to be, in general, a tendency all the way through for the immature frosted samples to increase in acidity at a slightly greater rate than the samples from normal wheat.

It is doubtful whether there was any increase in acidity of the kernels of the normal wheat stored at the lower temperature, however, a slight increase in the frosted sample is indicated. In the samples stored at room temperature, the normal sample of highest moisture content and apparently all three of the frosted samples increased in acidity, the frosted sample of highest moisture content increasing markedly. At 35°C. the same relation holds except that the increases are more marked.

The effect of grinding on the aging wheat was to accelerate the rate of change. In every case the sample with the highest moisture content showed a very marked increase in hydrogen-ion concentration and each of these also showed evidences of the growth of micro-organisms before the end of the experiment. As would be expected the growth of micro-organisms is evidenced first in the samples kept at the highest temperature.

The increase in hydrogen-ion concentration of the flour from the normal wheat of lowest moisture content for the three temperatures arranged in order of increasing temperature was 0.08, 0.10, and 0.13 pH units. While this increase might be considered small in comparison with some of the others, the interesting thing is that it took place at such a low moisture content. The increase in the normal wheat flour of intermediate moisture content is definite and is quite marked at 35°C. An extremely rapid change in hydrogen-ion concentration is noted in the high moisture content sample stored at 35°C. and a definite increase at the other temperatures. The effect of moisture and temperature is quite marked in the frosted samples. The increases occur in every case and are fairly uniform. A distinct difference

between the immature frosted and the normal sample is shown. The flour from immature frosted wheat increased in hydrogen-ion concentration at a faster rate than did the flour from normal wheat.

There is apparently a difference in the rate of change in hydrogen-ion concentration between the long patent and the low-grade flours from normal wheat. This is shown at the highest temperature where the low-grade flour increases in acidity the faster. The relationship is apparently the reverse with the flour from the immature frosted wheat, as in this case the long patent flour seems to have increased in acidity the faster. However, the low-grade flour samples from the frosted wheat had a lower moisture content, which may account for the difference.

The sample of low moisture content of the shorts showed no appreciable difference in rate of change in hydrogen-ion concentration from the long patent flour. The other two moisture content series increased more rapidly in acidity than did the flour. The shorts from immature frosted wheat increased in acidity less rapidly than did the patent flour altho this difference is pronounced only at the highest temperature.

The difference in rate of change in acidity between the bran from normal wheat, as shown in Table VIII and the long patent flour is apparent in only two cases, in which the samples of bran with the intermediate moisture content change faster than the corresponding samples of flour. These samples of bran, however, have a slightly higher moisture content. The samples of bran from the immature frosted wheat changed in acidity at a slower rate than did the long patent flour. There is an apparent horizontal portion in the curve for the frosted wheat bran with the intermediate moisture content at the highest temperature, the moisture content of the sample at the end of the year was found to be 9.4 per cent, perhaps due to a loosening of the stopper.

These results show that the long patent flour from normal wheat changes in pH with age at only a little slower rate than do the other mill products. Whatever may be the mechanism, it apparently tends to parallel the buffer content. The results obtained by Bailey and Peterson (6) and by Bailey (3) show that this buffer effect of flour is related to the ash content. The higher the ash content the more acid or alkali is required to produce a definite change in acidity. Assuming the relation of ash to buffer value, it would require more acid to change the hydrogen-ion concentration of bran through a definite interval than it would to change the patent flour. Thus, while

a greater amount of the outer portion of the endosperm in the flour stream would tend to add to the flour the factor which produces the increase in hydrogen-ion concentration on aging, at the same time buffer materials are added roughly, the proportion to counteract any pronounced acceleration in the rate of increase in acidity. The inclusion of greater amounts of the outer portion of the endosperm in the flour also tends to give a flour with a higher pH value, thus increasing the amount of acid necessary to bring such a flour to pH 5.0. The immature frosted wheat behaves somewhat differently in this respect, the long patent flour apparently increasing in acidity more rapidly than any of the other mill streams studied. It is not known whether this difference should be attributed to the immaturity of the sample or to the frost.

It might be argued that the mechanism which tends to increase the hydrogen-ion concentration of mill products from normal wheat is less active in the bran than in the patent flour of normal wheat, if we express the acidity in terms of actual hydrogen-ion concentration. Thus in going from pH 6.60 to 6.40 we have a decrease in the actual normality of hydrogen ions of approximately 0.14×10^{-6} while in going from a pH 6.20 to 6.00 the amount is much greater, 0.39×10^{-6} or more than twice as much acid is required to change the acidity in the latter case. This consideration does not take into account the buffer effect of the electrolytes present in the actual products and the range in pH where they exert their greatest effect. The results obtained by Bailey and Peterson (6) on the buffer effect of flour extracts indicate a straight line relationship, when the pH produced is plotted against the cc. of acid or alkali added to the flour extract. Their results thus indicate that the same quantity of acid would produce the same unit pH change within the range of pH found in the aging studies reported here.

If the increase in hydrogen-ion concentration produced by aging causes an actual improvement of the flour, then these experiments indicate at least two factors governing the acceleration of this change, i.e., high temperature and high moisture content. The use of a high moisture content of the flour as a means of accelerating the increase of hydrogen-ion concentration with age is, however, limited by the moisture content at which the growth of micro-organisms will take place in noticeable amounts.

The investigations reported here tell us nothing as to the effect of these conditions of aging on the other properties of the flour and wheat, which determine its baking value to a greater extent than

supposedly does the hydrogen-ion concentration of the flour. Experiments are now in progress to determine the effect of conditions of aging on other factors.

The amino nitrogen content of the samples reported on here was determined at the beginning and at the end of the year, but no differences could be detected.

Summary and Conclusions

1. The high-grade flour from normal wheat has a higher hydrogen-ion concentration than either the wheat or its other mill products.
2. The hydrogen-ion concentration of wheat, flour, and mill products increases with age under favorable conditions of temperature and moisture content.
3. The hydrogen-ion concentration of ground wheat increases more rapidly than that of unground wheat.
4. Results indicate that the flour from the sample of immature frosted wheat increased in hydrogen-ion concentration at a faster rate than did its other mill products.
5. Wheat and mill products of immature frosted wheat increased in hydrogen-ion concentration at a faster rate than did those from normal wheat.
6. It is not known whether these differences between immature frosted wheat and normal wheat were due to the effect of frost or to the immaturity of the sample.
7. High temperature and high moisture content accelerate the increase in hydrogen-ion concentration within the limits investigated.

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STUDIES ON WHEAT FLOUR GRADES IV. CHANGES IN HYDROGEN-ION CONCENTRATION AND ELECTROLYTIC RESISTANCE OF WATER EXTRACTS OF NATURAL AND CHLORINE TREATED FLOUR IN STORAGE[1]

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In an earlier paper[2] it was shown that treating flour with chlorine effected an increase in the hydrogen-ion concentration, and a decrease in the electrolytic resistance of the water extract. Portions of the same patent and clear flour samples were treated in the laboratory with varying quantities of chlorine per unit of flour, and the pH of the extract diminished regularly with increasing dosages of chlorine. The patent flour was altered in pH more than was the clear flour by the same treatment, owing to the higher buffer index of the latter. The specific conductivity of water extracts was increased regularly by increasing the dosage of chlorine, and in this particular there was comparatively little difference in the alteration of the patent and the clear grade flour. These conclusions are supported by the data presented in Table I.

TABLE I

Effect of Varying Quantities of Chlorine Upon the Specific Conductivity and Hydrogen-ion Concentration of Patent and Clear Flour Bleached in the Laboratory

Patent flour			Clear flour		
Chlorine used per 100 grams of flour	Specific conductivity of water extract	pH	Chlorine used per 100 grams of flour	Specific conductivity of water extract	pH
cc.	$K_{30} \times 10^4$		cc.	$K_{30} \times 10^4$	
0	5.51	5.51	0	8.50	6.00
10	5.70	5.33	10	8.72	5.93
20	5.99	5.17	20	9.23	5.83
30	6.21	5.00	30	9.70	5.73
40	6.64	4.77	40	10.05	5.61
50	7.06	4.60	50	10.34	5.49
60	7.40	4.43	60	10.54	5.38

Through the courtesy of the Pillsbury Flour Mills Company, of Minneapolis, samples of natural or untreated patent flour and of the same flour treated with chlorine at the rate of 0.5 ounce per barrel, were secured at the A Mill on August 4, 1921. The ash content was 0.40 per cent. Each of the two samples was divided into two portions, one of which was stored in a mason fruit jar, and the other in an ordinary cotton flour sack. These were placed in a cabinet in the

[1] Published with the approval of the Director as Paper No. 477, Journal Series, Minnesota Agricultural Experiment Station.

[2] Bailey, C. H., and Johnson, Arnold. 1922. Studies on wheat flour grades III. Effect of chlorine bleaching upon the electrolytic resistance and hydrogen-ion concentration of water extracts. J. Assoc. Off. Agr. Chemists, Vol. 6, pp. 63-68 (1922).

laboratory, and opened at intervals to withdraw a small sample for examination. The four lots have been stored thus for thirty-two months. In Table II are shown the results of the determinations of hydrogen-ion concentration of the water extract at intervals during this period. It appears difficult to draw any conclusions relative to the effect of the container on the change in pH, as the differences are in one direction in the case of the natural flour, and in the other direction in the case of the chlorine treated sample. In all instances there is a steady decrease in pH of the flour with the lapse of time. The greatest change in this particular occurred during the first year, since which time the rate of change is lower. The extent of change is somewhat less in the chlorine treated samples than in the natural flours, altho the former have changed substantially in pH. It accordingly appears safe to conclude that chlorine treated flours may be expected to increase in hydrogen-ion concentration with the lapse of time, and that the enzymes or organisms which effect this change have not been completely inactivated by the treatment with chlorine.

TABLE II

HYDROGEN-ION CONCENTRATION (AS pH) OF FLOURS MILLED IN THE PILLSBURY A MILL ON AUGUST 4, 1921, AND STORED 32 MONTHS

Date of test	Natural or untreated flour		Flour treated with ¾ oz. of chlorine per barrel	
	Stored in mason jar	Stored in cotton sack	Stored in mason jar	Stored in cotton sack
	pH	pH	pH	pH
August 6, 1921	6.07	6.07	5.65	5.65
October 3, 1921	5.92	5.95	5.48	5.46
January 13, 1922	5.78	5.79	5.44	5.61
February 12, 1922	5.78	5.80	5.48	5.61
May 23, 1922	5.73	5.70	5.27	5.54
October 18, 1922	5.75	5.61	5.26	5.44
December 29, 1922	5.68	5.60	5.22	5.38
June 1, 1923	5.65	5.53	5.25	5.34
August 6, 1923	5.60	5.48	5.25	5.25
December 28, 1923	5.55	....	5.25	....
April 9, 1924	5.53	....	5.24	....

The specific conductivity of the water extracts (1 part flour to 10 parts water) of these flours was determined at frequent intervals over a period of five months. During this period the change in this property of the flour was insignificant, the variations in the results of twelve determinations with each flour being within the limits of experimental error. The average specific conductivity of the natural flours was 5.48×10^4, and of the chlorine treated samples 5.95×10^4.

In connection with collaborative studies conducted under the supervision of O. S. Rask, as associate referee on cereal foods for the Association of Official Agricultural Chemists, we were furnished with samples of natural and chlorine treated flours. These were milled

during the summer of 1921, and duplicate samples were immediately sealed in tight cans. The hydrogen-ion concentration was determined in one pair of samples on their receipt at this laboratory. The other pair was stored unopened in the laboratory until April 9, 1924, when their hydrogen-ion concentration was determined. The following were the results of these determinations, and indicate that both increased in hydrogen-ion concentration, but that the rate of change was greater in the natural than in the treated sample.

	pH
Natural flour	
July 20, 1921	6.00
April 9, 1924	5.55
Chlorine treated flour	
July 20, 1921	5.65
April 9, 1924	5.39

In order to determine the effect of the chlorine dosage on the change in pH on storing flour, another series of samples was prepared. These were straight grade flours, with an ash content of 0.57 per cent and a moisture content of 10.2 per cent as milled at the Minnesota State Testing Mill on November 8, 1921. Six dosages of chlorine were used, at the following rates per hour, and with a flour stream of 6 barrels of 196 pounds per hour: 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ounces respectively. These dosages were equivalent to the following quantity of chlorine per barrel: 0.42, 0.50, 0.58, 0.67, 0.75, and 0.83 ounce. A control or untreated flour was drawn at the time that the treated samples were milled. These were placed in mason jars and stored in the laboratory. Hydrogen-ion concentration determinations were made at intervals during the following 29 months, with the results shown in Table III. A steady decrease in pH occurred in each sample, but the extent of change diminished somewhat with increased dosages of chlorine. There seems to be a tendency in the direction of arriving finally at approximately the same hydrogen-ion concentration, this condition having been practically reached in all the samples by December 28, 1923.

TABLE III

HYDROGEN-ION CONCENTRATION (AS pH)[1] OF STRAIGHT FLOUR TREATED WITH VARYING DOSAGES OF CHLORINE AND STORED FOR 29 MONTHS

Date of test	Chlorine per barrel of flour, ounces						
	0.00	0.42	0.50	0.58	0.67	0.75	0.83
	pH	pH	pH	pH	pH	pH	pH
November 9, 1921	6.04	5.93	5.90	5.88	5.87	5.83	5.80
January 13, 1922	6.03	5.97	5.95	5.93	5.92	5.92	5.87
February 12, 1922	6.04	5.95	5.93	5.91	5.90	5.89	5.87
May 26, 1922	5.95	5.90	5.89	5.90	5.90	5.83	5.81
October 18, 1922	5.95	5.87	5.86	5.86	5.86	5.82	5.81
December 29, 1922	5.87	5.78	5.78	5.73	5.71	5.71	5.70
June 1, 1923	5.80	5.75	5.73	5.71	5.73	5.71	5.70
December 28, 1923	5.72	5.75	5.78	5.73	5.73	5.73	5.71
April 9, 1924	5.75	5.73	5.75	5.73	5.75	5.71	5.65

Specific conductivity of the water extracts of these flours was positively correlated with the chlorine dosage, an increasing conductivity resulting from an increasing dosage of chlorine. As in the case of the samples from the Pillsbury Mill, the conductivity did not change with the lapse of time. This is shown by the data in Table IV.

TABLE IV

SPECIFIC CONDUCTIVITY ($K_{30} \times 10^4$) OF WATER EXTRACTS OF FLOURS MILLED NOVEMBER 8, 1921, AND TREATED WITH VARYING DOSAGES OF CHLORINE

Date of test	Chlorine per barrel of flour, ounces						
	0.00	0.42	0.50	0.58	0.67	0.75	0.83
November 9, 1921.....	5.85	5.95	6.03	6.10	6.16	6.19	6.24
December 12, 1921.....	5.86	5.95	6.04	6.12	6.15	6.21	6.24
January 12, 1922.....	5.85	5.96	6.04	6.13	6.16	6.21	6.24

A third series of flours was secured at a later date, representing 5 flours of varying degrees of refinement, as produced at the State Testing Mill. These were milled March 10, 1922, and only one sample, the straight grade, was treated with chlorine. This received the usual dosage of ½ ounce of chlorine per barrel of flour. This treated sample, and the five natural or untreated flours were stored in the laboratory in mason fruit jars for twenty-five months, and hydrogen-ion concentration determinations were made at intervals during this period. These flours contained the following percentages of ash:

	Per cent
Third middlings flour	0.33
Patent	0.40
Straight	0.64
First clear	0.82
Second clear	1.37

As shown by the data in Table V, the pH of water extracts of these flours has decreased during this period, the greatest change occurring during the first year. Except in the third middlings flour, which is the most highly refined flour in the series, there is little difference in the change in pH of the several flours. While the titrable acidity of these samples was not determined, it is probable that it increased more in the lower or clear grades than in the patent and straight grades, but the higher buffer index of the clear grades prevented any greater change in hydrogen-ion concentration than was observed in the higher grades.

A large sample of the Pillsbury flour was extracted with ether, the ether vaporized at low temperatures, and the extracted material stored in a tight jar. It contained 12.63 per cent of moisture, being normal with respect to moisture content, and there was no odor of ether in the atmosphere above the flour in the jar. The hydrogen-ion concen-

tration of this extracted sample was not determined immediately after the extraction with ether, but was determined at intervals from May 23, 1922, to April 9, 1924, and no change could be detected during this period. All the determinations ranged between pH 5.95, and 5.98, which are within the limits of experimental error. It is not entirely clear from this study whether (a) the ether removed from the flour that material which contributes to the increasing acidity of a normal flour, or (b) the ether inactivates the organisms or enzymes instrumental in modifying hydrogen-ion concentration. It seems probable that the first alternative may be accepted as the explanation of the small change in pH of flour thus extracted with ether.

TABLE V

HYDROGEN-ION CONCENTRATION (AS pH) OF SEVERAL GRADES OF FLOUR MILLED AT THE STATE TESTING MILL ON MARCH 10, 1922, AND STORED FOR 25 MONTHS

Date of test	3d Middlings	Patent	Straight Natural	Straight Treated with Cl_2	1st clear	2d clear
	pH	pH	pH	pH	pH	pH
March 13, 1922.....	5.78	5.92	6.03	5.80	6.24	6.38
May 24, 1922.....	5.71	5.79	5.87	5.66	6.07	6.20
October 18, 1922.....	5.70	5.79	5.83	5.65	6.09	6.17
December 29, 1922.....	....	5.65	5.67	5.51	5.93	6.04
June 1, 1923.....	5.65	5.61	5.56	5.52	5.93	6.02
December 28, 1923.....	5.56	5.58	5.60	5.46	5.87	6.00
April 9, 1924.....	5.51	5.58	5.56	5.44	5.85	5.97

Summary

Treatment of flour with chlorine effects an increase in hydrogen-ion concentration, and specific conductivity of the water extract proportional to the dosage used in the treatment.

Natural flours, and flours treated with chlorine increase in hydrogen-ion concentration with the lapse of time when stored under ordinary conditions. The natural flours appear to change somewhat more in this respect than the chlorine treated flours. There seems to be a tendency toward a constant degree of acidity when natural flour and the same flour after treatment with chlorine are stored, although it may require several years to reach the same pH.

Specific conductivity of water extracts remained constant, when extracts of a flour stored for varying lengths of time were compared.

Patent or high-grade, and clear or low-grade flours changed in hydrogen-ion concentration at about the same rate with the lapse of time.

Flour extracted with ether and dried, changed inappreciably in hydrogen-ion concentration when stored for nearly two years.

A STUDY OF THE ALEURONE CELLS OF CEREALS

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This research on the aleurone cells of cereals was carried out by the author because of the many and devious statements which appear in all textbooks on breadmaking and its kindred subject of flour milling. The facts recorded, very few in number and many apparently based on mere supposition, seemed to call for an investigation to be made on scientific lines.

When the study was begun, reference to available literature showed that all the work previously carried out with regard to aleurone grains and cells did not refer to cereals, but mainly to lupins, conifers, sunflower and hemp seeds.

Thorpe in his "Dictionary of Chemistry" gives the following facts: "The aleurone grains are said to be organized granules deposited in the cells of many seeds of plants generally near the exterior in which proteins are mainly concentrated. These were named by Dr. T. Hartig. In many plants they possess the shape of crystals, and botanists regard them as consisting of two parts: (1) Crystalloid (proteid), (2) one or more rounded globoids mainly composed of mineral matter in which phosphoric acid, lime and magnesia are usually the largest constituents. When the grains exhibit the form of crystals, it is generally found that they are soft and swell up when treated with weak acids or alkalis. The term crystalloid as used by botanists in this connection refers to the appearance, not to their diffusibility through a membrane when in colloidal solution."

Tschirch and Kritzler consider the proteid to be globulin, also that the grains and enclosures are reserve food material for consumption when the seed germinates. In 1879, S. H. Vines read a paper before the Royal Society on the "Chemical Composition of Aleurone Grains of Lupin Seeds," in which he showed that there was a small amount of globulin present. Also he obtained reactions characteristic of peptones.

M. S. Posternack, in 1900, published in Comptés rendus a paper on the "Chemical Composition and Significance of Aleurone Grains." He examined the following seeds: Sunflower, hemp, white lupin, and sapin rouge. He paid special attention to the conception that the aleurone grains furnished the reserve nitrogenous material. He found that they contained in round numbers 50 to 75 per cent of nitrogenous

material, 25 to 50 per cent of their weight constituting substances of mineral or organic origin. Posternack isolated a complex substance, an organic compound of phosphorus called by him "phytin." He states, as a result of this work, that aleurone grains not only appear as reserve nitrogenous material, but as a complete mineral food reserve for the plant embryo.

J. Percival, in his monograph on the wheat plant, says that the aleurone layer in wheat consists almost entirely of a layer one cell thick, measuring 65-70μ across, except that in the furrow and occasionally at other parts there are two or more cells superimposed. The cells are rectangular in the transverse and longitudinal sections and polygonal with round corners in the surface view, the diameters are 25-75μ. In front of the scutellum the layer consists of smaller cells, while over the plumule it becomes so thin and shrivelled that it is difficult to recognize it as a separate layer.

Formerly the cells were termed "gluten cells," but Schunk, in 1872, found that they contained no gluten. In each cell is a round or oval nucleus about 12μ in diameter and possessing three or four nucleoli. The rest of the cell cavity is filled with minute spherical bodies usually described as aleurone grains imbedded in a small amount of waxy or oily cytoplasm.

Each grain measures 3 to 4μ in diameter and consists of a highly refractive core surrounded by a thin membrane. When treated with water, salt solutions, weak acids and alkalis, the core dissolves, leaving the insoluble membrane as a hollow ball.

Strasburger in his textbook on botany states that globulins are present in the aleurone cells combined with calcium and magnesium salt of an organic phosphoric acid. Frequently calcium oxalate is present but in cereals, according to Strasburger, the aleurone grains are small and free from all inclusions and contain neither crystals nor globoids.

In the research work barley and wheat were the cereals employed, these being most important to the breadmaking and flour milling industries.

Part I. Isolation of Aleurone Cells from Different Cereals

At the beginning of this work Chevalier barley was placed in distilled water at 35°C. and allowed to stand twenty hours. Sections were then obtained, using a microtone and a sharp section cutting knife.

By the microscopical examination of sections of barley and later wheat, maize, rye, and buckwheat, the position of the aleurone cells

was definitely established, and the possibilities of obtaining them free from the endosperm considered.

With barley it was found possible to strip off the husk and then remove the aleurone layer, which was examined under the microscope. This method of "stripping" was carried out, periodical examination of the layer being made to ascertain that the correct layer was being removed. It proved very satisfactory with barley, as the barley kernels, being of a fairly hard nature, did not pulp on contact with water for a long period.

The liquors obtained from the barley by "steeping" were examined and many bacteria were found to be present, among them "tetanus bacilli" in large numbers in a very active condition. In all subsequent work the barley had to be sterilized after steeping, and the best method was by using a sterilizing solution of sodium chloride and mercuric chloride, application of heat not being suitable or effective owing to the gelatinization of the starch granules.

The aleurone layer obtained was kiln dried at 40°C. for three hours, as obtained. Eventually 15 grams were obtained which were utilized in the succeeding work.

The next cereal considered was wheat, the variety used being white durum, on account of its harder structure; as very early, difficulties were encountered because of the different physical structure and behavior of the wheat berry with regard to moisture content.

Durum wheat was soaked for six hours in tap water, then sections were cut and examined. An attempt was next made to strip off the aleurone layer, but the wheat berries were not sufficiently steeped. After a further six hours, the stripping could be done. A series of experiments was next made to examine the effects of different waters on the wheat berry when in steep, in order to see if certain waters would toughen the bran and hence facilitate removal, also cause the aleurone layer to be removed more easily.

1. *Permanent hard water* made by suspending 100 grams of calcium sulphate in 400 cc. of distilled water for twelve hours. With durum wheat a slight toughening of the bran was noticed but the aleurone layer was not readily removable.

2. *Temporary hard water,* made by passing gaseous carbon dioxide through 140 cc. of lime water, until the precipitate so formed was dissolved. In twelve hours a slight toughening of bran was noticed but the aleurone layer was removed only with difficulty.

3. *Ammonia-free water.*—With durum wheat in twelve hours contact, the degree of absorption obtained was not as great as in previous experiments.

4. *Distilled water* gave the same results as ammonia-free water.

5. *Manchester tap water.*—Best results were obtained, moisture penetrating to a greater extent the aleurone layer, allowing its removal with greater ease.

It was found possible to keep the moistened berries only one day and that by pouring off supernatant liquor; as after this period saturation causes the endosperm to become a soft, starchy, glutenous mass and hinders the removal of the aleurone layer.

As color is conferred on wheat by the testa, a white variety was used, as the testa could not always be removed separately. Forty-seven grams of this aleurone layer were obtained.

Time did not allow of the separation of large quantities of the aleurone layer from the other cereals, and so all further work described refers to the chemical examination of the aleurone layer of barley and wheat only.

Part II. Examination of Aleurone Layer from Barley

Experimental—Proteids and other matter soluble in water.—The material obtained by stripping was now utilized. An aqueous extract of any seed is in reality a saline solution, owing to the salts extracted from the seed; and as proteid matter soluble in alcohol is soluble to a very slight extent in very dilute saline solutions, the proteids properly soluble in water were obtained by extracting the aleurone cells with sodium chloride solution and dialyzing the salts away.

The aleurone cells were accordingly treated with 10 per cent sodium chloride solution in each experiment (5 grams in 250 cc. of 10 per cent sodium chloride solution) and maintained at a temperature of 20°C. for twenty-four hours. At the end of this time the extract was filtered clear and saturated with ammonium sulphate, when a moderate precipitate was obtained. This precipitate was filtered off, and redissolved in 10 per cent sodium chloride solution. All of the precipitate, however, did not redissolve, so it was now filtered off and the filtrate placed in a dialyzer in running water for seven days. At the end of this time the liquors in this dialyzer were tested for the presence of chlorides and sulphates and negative results were obtained. The precipitated matter was removed from the dialyzer and reserved for examination.

The clear solution obtained by filtration of the liquors from the dialyzer were now heated carefully in a water bath at 65°C. for two hours, when a slight coagulum was obtained. It was impossible to separate the coagulum by filtration, as it entered the pores of the filter paper, so it had to be separated by settling and decantation of the liquors. It was well washed with distilled water and dried in a vacuum desiccator over calcium chloride.

It was impossible to carry out an analysis of this product as such a small amount was obtained.

Extract from sodium chloride solution.—It is a difficult matter to prepare a globulin in anything like a pure condition because the globulin readily passes into an insoluble condition and this difficulty was encountered. Eventually after much treatment with alcohol and ether, and drying in a vacuum desiccator 0.3 grams were obtained of sufficient purity to be examined. Combustions were carried out and the following results obtained:

(1) 0.0556 grams taken
C—11.70%
H— 4.68%
N—12.44%
Ash—31.50%

(2) 0.0606 grams taken
C—12%
H— 4.7%
N—12.38%
Ash—32%

Extract obtained with dilute alcohol.—The residue of aleurone layer from the sodium chloride extraction was washed thoroly with distilled water until free from all chloride, then treated with alcohol, to make with the water retained by the residue a solvent of 75 per cent alcohol. Five grams were taken and digested with 500 cc. of alcohol for twenty-four hours at 20°C.

The extract was filtered clear and was then concentrated to a small bulk on a water bath, and the residue which separated was washed thoroly by kneading with distilled water. This was a dark greenish brown mass which was redissolved in alcohol at 50°C. A small amount, however, was not dissolved and this was separated by filtration. The clear extract was poured into absolute alcohol when a considerable precipitate was obtained.

By fractional precipitation a considerable quantity of protein was obtained which was dried and combustions were carried out on it.

(1) Weight of substance taken—0.0464 grams. C—54.32%
H— 6.81%

(2) Weight of substance taken—0.035 grams. C—54.20%
H— 6.67%

Nitrogen determined using 0.0531 grams. N—17.38%
S— 0.78%

Complete analysis: C—54.26%
H— 6.74%
N—17.38%
O—20.85%
S— 0.78%
Ash— 1.04%

A small amount of residues of a different character were examined. Amounts used for combustion (1) 0.0444 grams, (2) 0.0321 grams, (3) 0.031 grams.

Approximate composition: C—45.6%
H—11.23%
N— 7.37%
O and S—25.72%
Ash—10.08%

This ash was examined and was found to contain the following: A small quantity of manganese, sodium, potassium, calcium, and magnesium, sulphate and phosphate, as well as silica.

Conclusions.—The examination of the aleurone layer of barley revealed the presence of:

1. A small amount of protein, soluble in water, coagulated between 60-65°C., probably leucosin, and a small amount of proteoses.

2. A considerable amount of an amorphous mass, soluble in saline solution, precipitated by dialysis, approximate composition:

C—11.85%
H— 4.69%
N—12.41%
S— 1.17%
P— 8.15%
O—29.98%
Ash—31.75%

This was not of a homogeneous nature, probably consists of a mixture of proteid matter with aleurone grains and mineral salts of organic bodies.

The amount of phosphorus seems to indicate the presence of anhydro-oxymethylene diphosphoric acid—as suggested by Posternack.

3. Protein soluble in 75 per cent alcohol.

Approximate composition: C—54.26%
H— 6.78%
N—17.38%
S— 0.78%
O—20.8%

also another substance possessing a fibrous structure when examined microscopically.

Approximate composition: C—45.6%
H—11.23%
N— 7.37%
O—25.72%
Ash—10.78%

Traces of fatty matter were also observed in alcoholic extracts which were removed by treatment with ether.

Part III. Examination of Aleurone Layer from Wheat

The material obtained from the wheat by stripping was treated in a similar manner to that obtained from the barley, treated with 10 per cent sodium chloride solution at 14°C. for twelve hours (taking for each extraction 10 grams aleurone cells in 250 cc. solvent), the filtrate saturated with ammonium sulphate, and the resulting precipitates redissolved in 10 per cent brine and dialysed in running water for twelve days. At the end of this time the contents of the dialyser were transferred to a beaker and the precipitate allowed to settle. The clear liquors were heated to 65°C. in a water bath for one hour, the temperature of the water bath not exceeding 68°C., when a very faint precipitate was obtained. The slight coagulum was removed by filtration but was lost in the pores of the filter paper, and the resulting liquors were boiled for one hour, when a slight coagulum was obtained. This was separated and the liquors were evaporated down to dryness when crystals of aleurone grains were obtained along with other crystals later found on examination to be calcium oxalate, with traces of ammonium sulphate crystals.

A special extraction was carried out with 5 grams of aleurone cells and 200 cc. of distilled water at 20°C. for twenty-four hours. At the end of this time the extract was filtered clear and then boiled to coagulate any globulin present. A slight coagulum was obtained which was filtered off, and boiling was continued until no more turbidity appeared.

The clear solution was then evaporated to a small bulk, and filtered into absolute alcohol, when a dense white precipitate was obtained. This precipitate was readily soluble in distilled water and this solution gave no turbidity on boiling. This gave the xanthoproteic and Millon's reactions, a precipitate with potassium ferrocyanide and acetic acid, and a faint coloration with excess caustic soda and one drop of very dilute copper sulphate solution (0.1 per cent solution being used). These properties and reactions indicate similarity to peptones, and resemble properties of a substance obtained from blue lupin by S. H. Vines, but most readily resemble "Hemialbumose of Kuhne."

Sodium chloride extract.—The precipitates from the dialyses were well washed with distilled water and dried in a steam oven at 98°C. for three hours, and were then subjected to analysis, the following results being obtained:

(1) Amount taken—0.0393 grams. C—23.6%
H— 5.09%
Ash—23.74%

(2) Amount taken—0.0438 grams. C—24.2%
H— 5.2%
Ash—23.6%

(3) For nitrogen determination, amount taken—0.062 grams,
N—13.18%

(4) For sulphur determination, amount taken—0.0312 grams,
S— 1.68%

Extract by means of dilute alcohol.—The residue from the sodium chloride extract was washed thoroly with distilled water, till the wash water was free from chlorides. The residues from each extraction were treated with 300 cc. 75 per cent alcohol for twenty-four hours at 20°C., and at the end of this time the extract was filtered clear.

Several of the residues were treated with alcohol at 55°C. for eight hours and another portion was refluxed with alcohol for eight hours and the extracts filtered clear.

The extracts obtained at 20°C. were concentrated to a small bulk under reduced pressure when a brownish residue was obtained which was thoroly washed by kneading with water. This was added to dilute alcohol, and nearly all dissolved, the residue being separated by filtration. The solution was then added to a large volume of absolute alcohol, but no precipitate of protein resulted.

This solution was then evaporated to smaller bulk, and shaken in a separating funnel with ether, when the alcoholic layer was withdrawn and added to absolute alcohol, but no precipitate resulted. The ether was evaporated off from the ethereal layer, and a brownish, oily residue was obtained; this was redissolved in ether, treated with animal charcoal, and filtered, when a pale yellowish oil was obtained which on examination had a refractive index of 1.471.

The alcoholic extract was shaken with ether again, and then poured into absolute alcohol, and this process was continued four times when the final extract was evaporated to dryness and a brown powder resulted. During one experiment a brownish residue underwent a change while being treated; the brown powder changing, during a kneading operation, to a plastic fibrous mass which on cooling changed to a horny mass and darkened in color on being kept.

The extraction made with alcohol at 55°C. had a distinct rancid odor and on evaporation under reduced pressure deposited a brownish mass on the side of the flask with a fatty appearance, along with other

matter on the bottom of the flask. This brownish mass was removed from the sides of the flask and treated with ether and chloroform, and a considerable amount of lustrous yellowish flakes was obtained. The residue from the bottom of the flask was treated with alcohol and after much treatment a product was obtained which was precipitated by pouring into absolute alcohol. This was collected and dried in a vacuum desiccator. The oil obtained from the ether extracts was purified by treatment with animal charcoal and some white, fatty solid was obtained which on purification melted at 136°C. This was suspected of being phytosterol, so a mixed melting point was carried out.

Melting point of phytosterol, 137°C.

Melting point of mixture of phytosterol and substance, 137.6°C.

Melting point of substance (after further purification), 138°C. This was accordingly considered to be phytosterol.

The alcoholic extract made by refluxing the aleurone residue with alcohol gave a small amount of protein, but much fatty matter, and lustrous flakes similar to those obtained from 53°C. extraction.

Combustions were carried out on (1) lustrous flakes, (2) substance resembling protein obtained from alcoholic liquors by evaporation.

It was impossible to carry out combustions on some of the products obtained owing to the small quantity. In all the work done very small quantities were employed, 0.04 to 0.06 grams being used.

(1) Lustrous flakes (a) amount taken—0.061 grams,
C—45.85%
H— 6.4%
Ash—10.8%
(b) amount taken—0.0526 grams,
C—45.67%
H— 6.5%
Ash—10.4%

Nitrogen determined on 0.0776 grams gave 16.83%.

Ash contained sulphates and phosphates, iron, aluminum, sodium, potassium, calcium, zinc, magnesium.

(2) a. Amount taken—0.038 grams, C—24.27%
H— 4.74%
Ash—44%
b. Amount taken—0.04 grams, C—23.875%
H— 4.61%
Ash—45%

The ash contained the same substances as above with the exception of iron.

Nitrogen from 0.04 grams—15.33%.

Micro-chemical examination of aleurone layer.—A section of the aleurone layer was placed in ether and alcohol to remove fat and water. This section was examined microscopically, showing the cells to be filled with aleurone grains in cells of a delicate matrix. They were hyaline or faintly granular and of a pale yellowish tint. On the addition of one drop of distilled water they became coarsely granular, the granules disappearing after being in water for some time, and vacuoles making their appearance.

A drop of 10 per cent sodium chloride solution was added and after a few minutes the hyaline vacuolation disappeared and nothing remained but a delicate matrix. Photographs of these changes were obtained.

Summary

The aleurone grains of cereals can be obtained in a crystalline form, together with crystals of calcium oxalate, thus disproving Strasburger's statement that in cereals they are not present as crystals.

The aleurone cells contain small quantities of protein, (a) soluble in water, (b) soluble in salt solutions, (c) soluble in 75 per cent alcohol, but only in very small quantities, too small for analysis. Small quantities of a greyish amorphous body, soluble in salt solutions, precipitated by dialysis, on analysis gave following results:

C—23.9%	S— 1.68%
H— 5.15%	O and P—32.42%
N—13.18%	Ash—23.67%

In the aleurone cells of barley only a small amount of fatty matter is present; but in the aleurone cells of wheat there is a large amount of wheat oil, refractive index 1.473, also a white waxy substance, probably phytosterol, melting point 138°C. Too small an amount of the last was obtained, however, for an acetyl value to be determined.

In the aleurone cells of wheat, a protein body soluble in water but precipitated by absolute alcohol was obtained. This resembled in reactions the substance obtained by S. H. Vines, and termed "Hemi-albumose."

A considerable amount of a lustrous flaky mass was obtained, giving the following results on analysis:

C—45.76%	O and S—20.66%
H— 6.45%	Ash—10.3%
N—16.83%	

This was a residue from alcoholic treatment, very slight precipitates of protein matter being obtained by precipitation by absolute alcohol.

A residue from alcoholic liquors gave on analysis:

C—24.07%	O and S—11.4%
H— 4.7%	Ash—44.5%
N—15.33%	

A small amount of a plastic fibrous mass was obtained, resembling coagulated protein, and under an eye-piece resembling beeswax.

The total nitrogen in the aleurone cells was determined by Kjeldahl's process and found to be 2.7 per cent, equivalent to 16.875 per cent of protein (Factor N×6.25).

In only few cases did the alcoholic extract yield any trace of protein by precipitation with absolute alcohol, even after prolonged treatment with alcohol and ether, which seems to indicate that protein may exist in the aleurone cells as such but is surrounded by fat particles which act as a protective colloid and so impede precipitation, the small amount of phytosterol present tending to keep the particles of fat and protein in an emulsoid state.

These observations also suggested to the writer the close relationship between fats and proteins which may be present in chemical combination, or may occur in the seeds of plants, a product which is a transformation product of fats to protein, rather than protein to fat.

The mineral residues obtained from all combustions contained the following mineral substances: calcium, magnesium, sodium, potassium, iron, aluminum, zinc, and in several cases manganese, which is stated to be an essential food factor of phanerogams by Bertrand, later confirmed by Bottomley.

The residue from alcoholic extraction on analysis was found to have a high mineral residue and nitrogenous content and is considered to be a "vitaminic" extract, as it resembled a product obtained from bran by similar methods and stated to be a vitaminic extract, and this would suggest that the presence of vitamines in bran is due in part to the aleurone layer. Finally the author desires to draw attention to the small amounts of products obtained, small amounts used for combustion and consequent difficulty in doing series of experiments apart from those mentioned.

In conclusion I desire to acknowledge my deepest gratitude to Mr. James Grant, J.P., M.Sc., Tech. F.I.C., etc., head of Foodstuffs Department, Faculty of Technology, Manchester University, under whose supervision this work was carried out, for his constant guidance and assistance during its execution.

THE SIZE OF THE PORES IN BAKED BREAD

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Translated from the German by Charles G. Ferrari

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The distribution, uniformity, and size of the grain cells in a loaf of bread play an important part in judging the capacity of a flour for baking.

As an individual factor in the baking qualities of a flour, the form and size of the pores are influenced by various causes, just as the flour itself is influenced.

The variety, the source and harvest conditions of the wheat, climate and fertilization, wheat blending and milling, the fermentation and manipulation of the dough, are all conditions definitely influencing the pore structure of bread. The degrees of difference in size of the cells of the loaf are just as numerous as these influences.

In exact, scientific baking experiments flours were baked comparatively with accurate checks under similar experimental conditions. In this manner a series of influences were eliminated, especially those of fermentation and manipulation. In spite of this the influence of the remaining chief factors led to a great difference in the structure and size of the cells.

In an experimental baking report, or in rendering an opinion of the baking capacity of a flour, it is very difficult to characterize in words the nature of the bread pores.

In describing two extreme cell structures, one writes for example, "The cells are very evenly distributed and very fine," or "The grain shows a very irregular cell structure, and the individual pores are very large." These words permit the reader a wide range of interpretation. If more than two flours are baked in comparison with one another, then it becomes especially difficult to define exactly by means of words the size of the cells of the various loaves.

The baking capacity of a single flour can be increased by a change in its enzyme strength, in the hydrogen-ion concentration of a dough made from it, in other words, through the addition of various substances. It is quite important to establish through baking experiments by what changes in the determining physical constants of flour (and of the later dough) the optimum baking capacity can be obtained. It has been demonstrated that the change of hydrogen-ion concentration has a direct, important influence upon the refinement of the grain. In these cases it is especially difficult to express in words the fineness

and the degree of difference obtained in the improvement of cell size, if the normal flour is subjected to several different treatments and the flours are baked side by side.

The best method for illustrating the results of baking experiments will always be photography. I have already described in Volume 3, No. 8, of "Zeitschrift für das gesamte Getreidewesen" a method designed to replace the tedious and often unfeasible photograph by a simple impression of the cell structure. The proposed method even has the advantage of reproducing the cells in natural dimensions. A slice of bread as flat as possible, or a similarly cut piece of a baked loaf, after blacking with a paste-like mixture of lampblack and oil, is pressed exactly like a stamp upon a piece of unsized paper. The resulting impression retains in its natural form the grain cell structure.

This process still has the objection that these reproductions must be attached to the report, and that generally more than one unobjectionable impression can not be made. For this reason, after collecting impressions of baked loaves over a period of time, I have compiled an ascending series of grain cells of ten sizes.

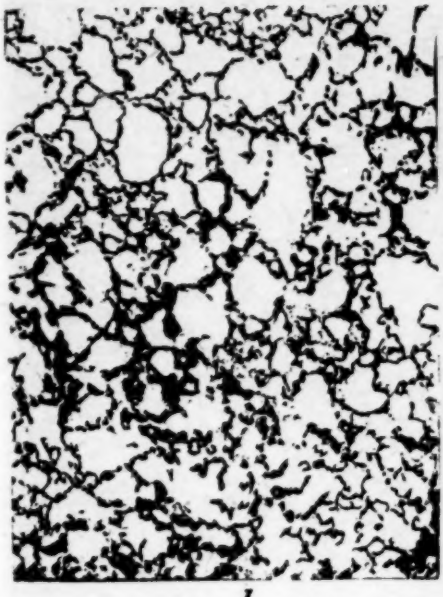
If one cuts an experimental loaf, the general cell size can be established by comparison with the proposed scale, and fixed numerically by specifying a number. This has the advantage that every research or flour laboratory carrying on experimental baking can express grain cell size simply by means of a number in the written report of a comparative baking experiment, just as it already does in the case of dough yield, bread yield, or the volume.

By stating the cell size of bread through the use of a number from 1 to 10, every one who is in possession of the grain cell scale will be able to form, on inspection of the correspondingly numbered illustrations on the scale, an exact picture of the general appearance of the grain, both immediately and at a future time.

No long, well intended explanation of the size of the cells will be given in a report in the future, but simply a number between 1 and 10 will appear which expresses all that is necessary.

The scale also serves the miller as a further aid in the classification of his flours.

For the purpose of increasing the baking capacity by the indicated procedure, an improvement of the grain in individual flours of four units can be obtained. In other words, a flour in which the cell size of the loaf was established, for example, as 3, showed after correct manipulation a cell size of 7. Improvements of this sort in the baking capacity of flours are not out of the ordinary. It is especially to be



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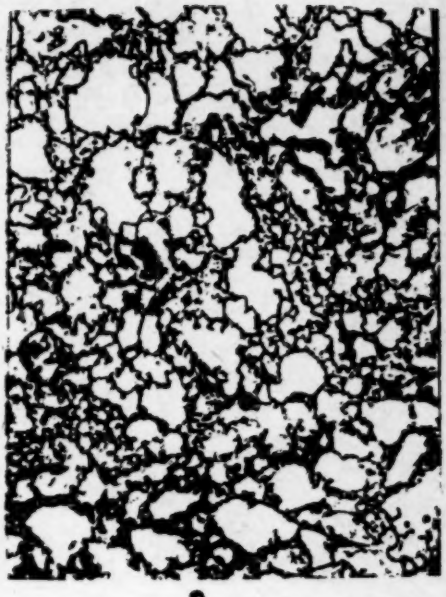

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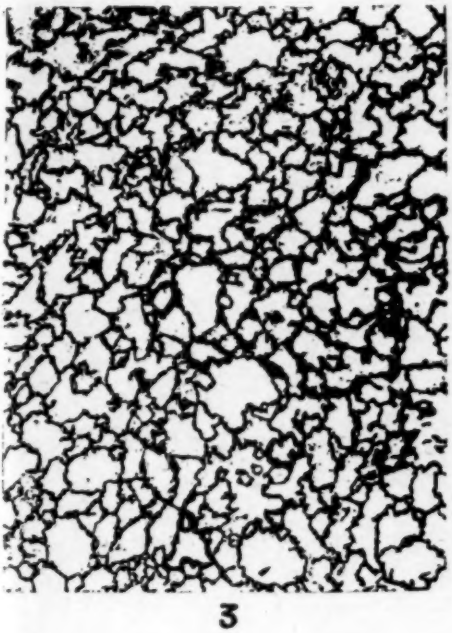

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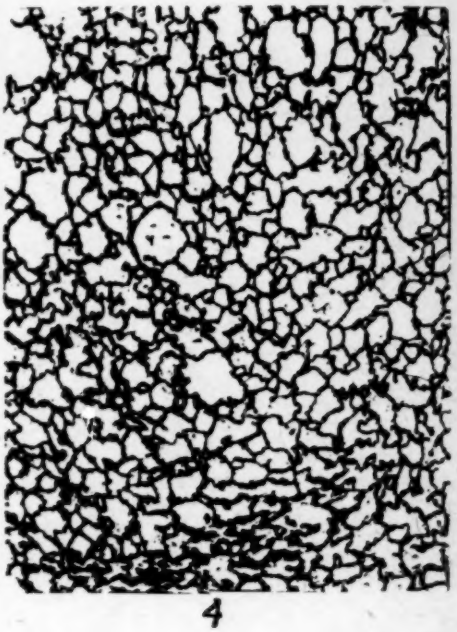

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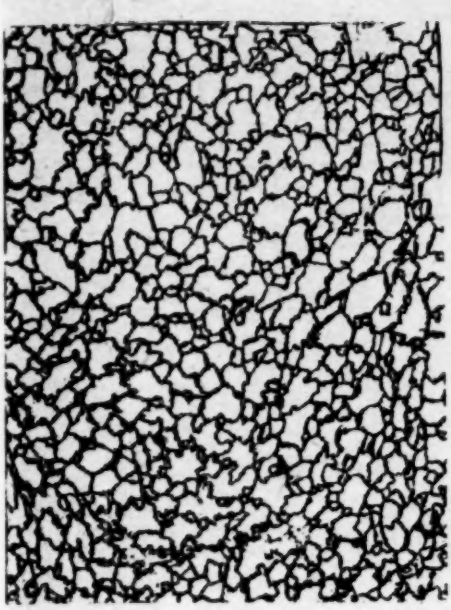

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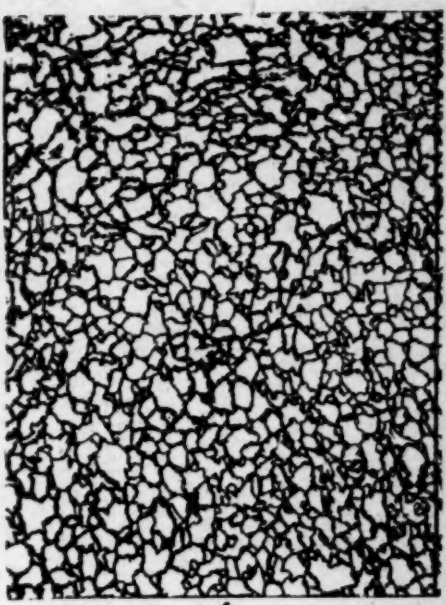

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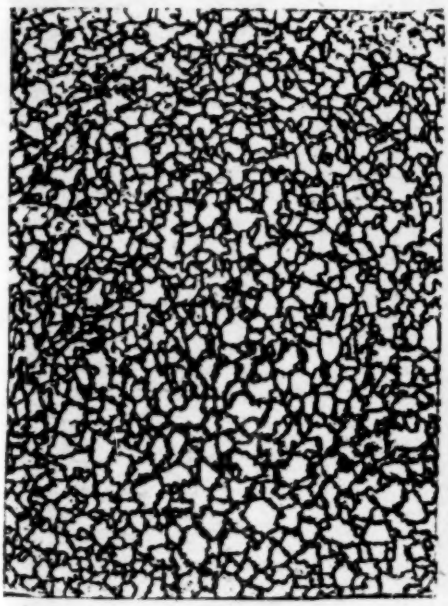

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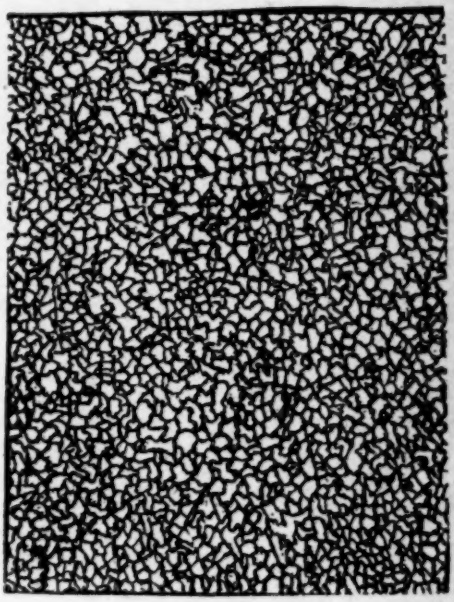

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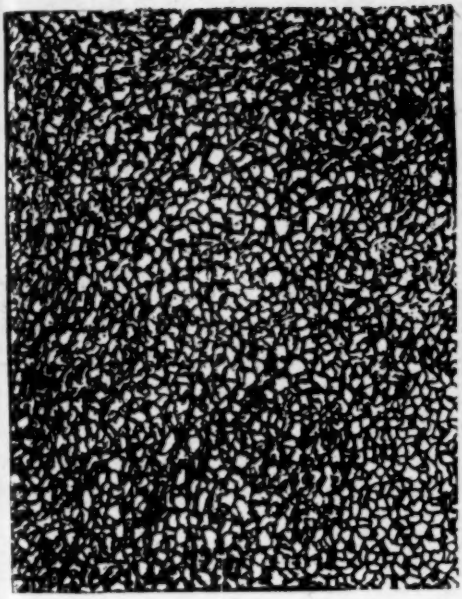

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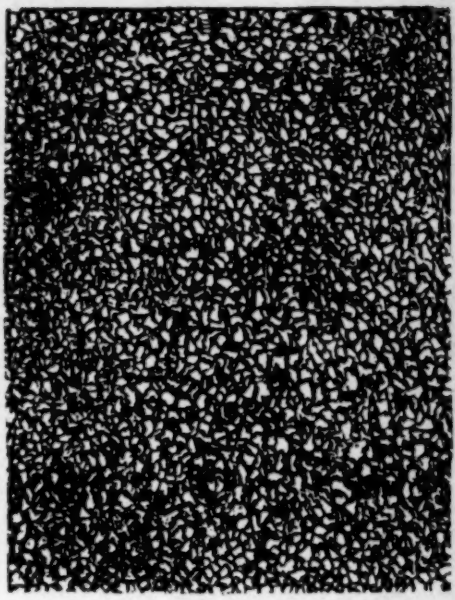

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